THE CULTIVATION OF *TRYPANOSOMA RHODESIENSE*

PRELIMINARY NOTE

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(*Received for publication* 2 *April*, 1912)

While doing research under the Sir Edwin Durning-Lawrence fund it was suggested to me by Sir Ronald Ross, that I might attempt to cultivate trypanosomes pathogenic to man, especially the new species of Stephens and Fantham (1910), namely *T. rhodesiense*.

After many failures success was attained by the use of a modification of the Novy-McNeal-Nicolle medium. In the water of condensation of this medium *T. rhodesiense* has been successfully cultivated for two weeks by incubating at a temperature of 25° C. to 28° C., and subcultures of one generation have continued to develop for eleven days, and then all flagellate forms disappeared. In this preliminary note I shall give as concisely as possible, the changes which take place in the culture tubes. It was found that these cultures were most successful if young trypanosomes were inoculated into the tubes. By young trypanosomes, I mean trypanosomes taken from an infected rat about the second day after their appearance in the peripheral blood.

In some of the culture tubes the development was rapid, and when such occurred the culture died out much more quickly, i.e., in about seven days.

The most remarkable feature about the cultures is the striking changes which take place in the trypanosomes. The trypanosomes become larger, and forms are found which are very similar to those described by Sir David Bruce and his colleagues (1911) in the gut of tsetse-flies fed on animals infected with *T. gambiense*. When the development is rapid, about the third day, the trypanosomes are seen to have become much larger, and on the fourth day two distinct types can be distinguished which resemble the so-called sexual forms

of Professor Kleine and Dr. M. Taute (1911) found in tsetse-flies fed on animals infected with *T. gambiense*. In cultures which developed more slowly, it was found that trypanosomes disappeared about the third and fourth days, reappeared about the sixth day, and on the eighth day spirillar forms were seen to be splitting off. The differentiation into the so-called male and female forms takes place during the eighth, ninth and tenth days.

In cultures of *T. rhodesiense* long, thin forms are found with a posterior nucleus, the nucleus being close up to the blepharoplast, and not behind it. Stout forms of large size corresponding to the female forms of Kleine and Taute have been seen. Many of these stout forms have very large nuclei, and in some their nucleus is posterior. Kleine and Taute, in their study of the development of *T. gambiense* in the tsetse-fly, figure numerous trypanosomes, both thin and stout, with the nucleus behind the blepharoplast; and Sir David Bruce and colleagues (1911) found slender forms which were crithidial, but only a very small percentage of these occurred in the intestine of *Glossina palpalis*. So far I have not found definite crithidial forms. When long, thin forms and stout forms are found in the culture together, I have often seen what might possibly be conjugation occurring between the so-called males and females in fresh as well as in stained specimens. Another very constant feature in cultures of rapid growth is the early occurrence of a multiple longitudinal fission of the trypanosomes into numerous thin or spirillar forms, very similar to that described by Leishman (1905) in the cultivation of *Leishmania donovani*.

In cultures in which development was slower, and which lived for fourteen days, it was found that *T. rhodesiense* seemed to disappear from the water of condensation for two or three days, and then reappeared. On such reappearance trypanosomes were observed as well as large plasmodial forms which evidently split up into numerous brood trypanosomes, and again the two distinct types, the so-called sexual forms of Kleine and Taute found in tsetse-flies, were seen.

From a study of these cultures I think that we may have a sexual phase in the life cycle of trypanosomes, and that in all probability we have occurring in culture tubes just that which takes place in the cycle of development of trypanosomes in the alimentary tract of

tsetse-flies after feeding on an infected animal. My chief reason for so thinking is that the trypanosomes found in cultures appear in forms very similar to those described by Kleine and Taute in the intestinal tract of tsetse-flies. It is, however, most difficult at the present stage of this research to make a definite statement regarding these forms, or to say what exactly occurs after conjugation, for at present what has been described as occurring in tsetse-flies is the development of *T. gambiense*, and as I have only studied *T. rhodesiense* in cultures it is impossible to compare the two morphologically, but the general behaviour of *T. rhodesiense* in cultures shows changes which in many respects resemble the development of *T. gambiense* in tsetse-flies.

During periods in which flagellate trypanosomes are very scarce or absent altogether from cultures, distinct rounded bodies have often been found like those described by Fantham (1911).

In the cultures obtained, there was undoubted multiplication of trypanosomes, as evidenced by the fact that in two cultures the flagellate trypanosomes entirely disappeared from the water of condensation of the cultivating media for at least two days, and again reappeared showing active division and becoming very numerous.

Division of trypanosomes was well seen in living specimens, and stained specimens showed division of nucleus and blepharoplast. Another feature noted in the cultures was the complete or almost complete absence of a free flagellum, which seemed to stop short at the anterior end of the trypanosome or only to project slightly beyond it.

So far, inoculation of animals from the cultures has been unsuccessful, which may be accounted for by the fact that the inoculations were possibly made before complete development of the trypanosomes had taken place, since we know that tsetse-flies after feeding only became infective after a certain latent period.

I hope to publish at an early date a full account of these cultures of *T. rhodesiense*, with illustrations of the different forms which occur. The trypanosomes employed to obtain these cultures were undoubtedly *T. rhodesiense*, since posterior nuclear forms were found in the blood before inoculation, and I have been able to exclude an accidental contamination of *T. lewisi* by using tame rats,

which were carefully examined before inoculation. In addition to this, a careful comparison has been made by growing *T. lewisi* as a control, and it is found that the cultures of *T. rhodesiense* showed no similarity to those of *T. lewisi.*

Finally, the modification of the Novy-MacNeal-Nicolle medium used in the cultivation of *T. rhodesiense* consists in substituting for defibrinated rabbit's blood, citrated rat's blood heated to 45° C. for half an hour, and sea salt was substituted for ordinary sodium chloride.

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